NEW MATHEMATICAL FORMULA FOR 
DIFFERENTIATING THALASSEMIA TRAIT AND IRON 
DEFICIENCY ANEMIA IN THALASSEMIA PREVALENT 
AREA: A STUDY IN HEALTHY SCHOOL-AGE CHILDREN

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Abstract. Iron deficiency anemia (IDA) and thalassemias are common diseases 
especially in the Mediterranean, Middle East and Asian regions. Both conditions 
show the same clinical findings of hypochromic and microcytic red blood cells. 
Although previous studies have devised mathematical formulae to differentiate 
between these two conditions, the prevalence of α- and β-thalassemias among 
the affected populations may undermine the accuracy of these formulae. This 
study generated a new formula that was able to differentiate IDA and thalas 
semia traits and to determine the incidence rates of IDA and thalassemia traits. 
A total of 345 healthy Thai children with a mean age (± SD) of 11.3 (± 1.7) years 
were enrolled. Complete blood count, iron status, hemoglobin typing and DNA 
for α-1 thalassemia identification were investigated. Discriminant analysis was 
used to create a new mathematical formula containing significant variables to dif 
ferentiate between IDA and thalassemia traits. The new formula of (1.5 Hb-0.05 
MCV >14) had a receiver operator characteristic curve of 0.92 in differentiating 
thalassemia traits from IDA, with sensitivity and specificity of 84.6 and 87.5%, 
respectively. The incidence of IDA and thalassemia traits in the study group was 
12% and 32%, respectively. This formula should be useful as a screening tool to 
differentiate between these two conditions.

Keywords: anemia, children, formula, iron deficiency anemia, thalassemia trait

INTRODUCTION

Iron deficiency anemia (IDA) is a common problem worldwide with an 
incidence of around 11%. In Thailand, the incidence of IDA is 1.5-8% (Winichagoon, 
2002; Panomai et al, 2010). According to World Health Organization (WHO) crite 
ria, laboratory diagnosis of IDA includes low hemoglobin (Hb) level for age, mi 
rocytic red blood cells (RBC), low ferritin level and/or low transferrin saturation
(Michaelsen et al., 2000).

Thalassemia is an autosomal recessive inherited hemolytic anemia, caused by a decrease in or abnormal production of α- or β-globin chains (Fucharoen and Winichagoon, 2011). The incidence of thalassemia trait is around 1.7% of the world’s population and the number of new cases of thalassemia is approximately 2.4 per 1,000 births (Angastiniotis et al., 1998). Thailand has a high incidence of thalassemia traits (20-25%) and the prevalence of diseased individuals is 1% (Nutrition Division DoH, 1995; Fucharoen et al., 1998a; Weatherall and Clegg, 2001; Thurlow et al., 2005). The most common thalassemia carrier is Hb E (up to 50%), followed by α-thalassemia trait (20-30%), and β-thalassemia traits (3-9%) (Wasi et al., 1980).

In a screening test of both IDA and thalassemia traits conducted using a complete blood count (CBC), RBC parameters may show similar results, namely low Hb and mean corpuscular volume (MCV) (Clarke and Higgins, 2000). In order to differentiate between these two conditions, additional laboratory investigations are required, such as ferritin, serum iron, total iron binding capacity (TIBC) levels, Hb typing and DNA analysis (for α-thalassemia trait), resulting in increased health care expenses. Previous studies employed a number of mathematical formulae (RDW/RBC and RDW indices; formulae of Bessman, Ehsani, England, Green and King, Mentzer, Shine and Lal, Sridah M, and Srivastava,) (Bessman and Feinstein, 1979; Ehsani et al., 2005; Eldibany et al., 1999; England, 1989; England and Fraser, 1973; Green and King, 1989; Lafferty et al., 1996; Mentzer, 1973; Ntaios et al., 2007; Ricerca et al., 1987; Shine and Lal, 1977; Sirdah et al., 2008; Srivastava and Bevington, 1973) in order to differentiate between IDA and thalassemia traits, usually based on various RBC parameters, but the majority of the formulae were employed to distinguish IDA from β-thalassemia trait.

In Thailand, using the formulae of Green and King, RDWI, Keikhaei and modified RDWI to distinguish between IDA and thalassemia traits produced a similar area under the curve of 0.73 (Wongprachum et al., 2012). However, with this value, it may not be sufficient enough to differentiate IDA and thalassemia traits. Therefore, this study established a new mathematical formula to differentiate between IDA and thalassemia traits, and to determine the incidences of IDA and thalassemia traits in school-age children in the central part of Thailand.

**MATERIALS AND METHODS**

**Study population**

Grade 3 to 9 students from an elementary school in Pathum Thani Province, central Thailand, were enrolled in the study. They had no underlying medical conditions or illnesses within one month before enrolment. Questionnaires, regarding the illnesses within one month, underlying diseases, food intake, history of blood loss, current medications and family history of anemia were filled in by students and parents. Detailed demographic data, such as age, sex, height and body weight were collected. Subjects with a history of infection or inflammation within one month before enrolment and those with underlying thalassemia diseases were excluded. The enrolled students were investigated for iron status and thalassemia.

Then they were classified as IDA, iron deficient erythropoiesis (IDE), iron depletion (ID) or iron sufficiency according to WHO criteria (Michaelsen et al., 2000).
IDA is diagnosed if the subject's Hb level is less than normal for age, transferrin saturation < 16% and/or serum ferritin level < 12 µg/l. IDE is defined by normal Hb level, transferrin saturation < 16% and/or serum ferritin level < 12 µg/l. ID is defined by a normal Hb level for age, normal serum ferritin and transferrin saturation.

The study was conducted during May 2008-April 2010. The study was approved by the Ramathibodi Hospital ethics committee and informed consent forms were obtained from the parents.

**Laboratory study**

Blood samples were collected in 2 tubes: ethylenediaminetetraacetic acid (EDTA) for testing of CBC using an automatic machine (Coulter JT) and Hb typing determined by high performance liquid chromatography (HPLC) using VARIANT II HPLC system (Biorad Laboratories, Hercules, CA) (Kirk et al., 2005), and clotted blood for serum for determination of ferritin by chemiluminescence (Abbott®) (Blackmore et al., 2008), and iron and total iron-binding capacity levels by colorimetric method (Huebers et al., 1987).

DNA was extracted from buffy coat using a phenol-chloroform method (Kan et al., 1977). Southeast-Asian deletional α-thalassemia (—SEA/), the most common α-thalassemia 1 in the region, was identified using a Gap-polymerase chain reaction as described previously (Sanguansermisri et al., 1999). Other types of thalassemia traits were determined according to the Hb typing results (Fucharoen et al., 1998a). Patients with Hb A₂ >3.5% and MCV<80 fl were diagnosed as β trait.

**Statistical analysis**

Data of subjects diagnosed with IDA and thalassemia traits with or without IDE and IDA were included for further analysis. Data analysis was performed using SPSS software, version 17 (IBM, Armonk, NY). Mean, standard deviation and percentage were used to describe the hematological data of study subjects. Data on red blood cell variables, namely, Hb, RBC, MCV, mean corpuscular hemoglobin (MCH), and red cell distribution width (RDW) were analyzed using discriminant analysis to create a new mathematic formula that contained significant variables to allow differentiation between IDA and thalassemia traits. Stepwise procedure was performed to test the statistically significant effects of those variables in a discriminant analysis process. Criterion for selecting the best formula was the lowest number of variables contained in the formula while providing high discriminating efficiency. The cut-off value was then obtained from the formula, which corresponds to the highest accuracy (minimum false negative and positive). In addition, the receiver operator characteristic (ROC) curves for various formulae which had been proposed including our new formula were constructed in order to calculate the area under the curve.

**RESULTS**

Three-hundred and forty-five healthy children, age (mean ± SD) 11.3 ± 1.7 years, and female : male ratio of 1:1.8, were enrolled in the study. Forty subjects (12%) were classified as IDA, 131 (37%) as IDE and 3 (1%) as ID. Thalassemia diseases and traits were found in 10 subjects (3%) and 111 subjects (32%), respectively. Seven out of 10 subjects were Hb EE, 2 Hb H disease and 1 was Hb E/β-thalassemia disease. The most common thalassemia trait was Hb E (19%) followed by α-thalassemia 1 (5%), β-thalassemia (4%),
Hb Constant Spring (CS) (3%), Hb E/CS (1%) and Hb E/α-thalassemia 1 (< 0.5%) traits. About 57% of IDA subjects had thalassemia traits. RBC parameters of normal, IDA, IDE, Hb E trait, and α- and β-thalassemia traits are shown in Table 1.

Hb and hematocrit levels, RBC number, MCV, RDW and platelet count in the IDA group were significantly different when compared to those in thalassemia trait group (Table 2). The median values (range) of serum ferritin level in IDA, 37.3 (2.1-162.4) µg/l, and in IDE, 34.3 (3-268.2) µg/l, groups were significantly lower compared to that of normal subjects, 46.9 (13.4-232.9) µg/l (p < 0.001). Serum ferritin level had an area under the ROC curve of 0.4 for the diagnosis of IDA.

In order to demonstrate that the formula was able to differentiate between IDA and thalassemia traits, 40 IDA subjects with or without traits were selected as the IDA group, while subjects with thalassemia traits with or without IDE or ID were selected as the thalassemia-trait group (Table 2). The previously reported formulae were used to calculate the areas under ROC curves (Fig 1) and the values of sensitivity and specificity are shown in Table 3.

The formula constructed from discriminant analysis for identifying thalassemia traits and IDA is (1.5 Hb - 0.05 MCV) with a cut-off value of > 14. Comparison of the performances of published formulae including our formula in differentiating thalassemia trait and IDA showed that our formula provided the highest area under the ROC curve (AUC) (0.92) (Fig 1) whereas RDW index provided the lowest AUC (0.34). The RDW/RBC index gave AUC of 0.81 while the remaining indices gave AUC < 0.6 (Table 3 and Fig 1). The new formula differentiated thalassemia traits from IDA with sensitivity and specificity of 84.6 and 87.5%, respectively (Table 3).

**DISCUSSION**

Although previous studies have used various formulae to differentiate IDA from thalassemia traits (England and Fraser, 1973; Mentzer, 1973; Shine and Lal, 1977; Bessman and Feinstein, 1979; England, 1989; Green and King, 1989; Lafferty et al, 1996; Eldibany et al, 1999; Ntaios et al, 2007; Sirdah et al, 2008; Wongprachum et al, 2012), most were retrospective studies of patients or pregnant women who came to the hospital. As such, the results may not indicate the incidence of IDA and thalassemia traits in the general population. In addition, most of the studies aimed to differentiate β-thalassemia traits from IDA (England and Fraser, 1973; Mentzer, 1973; Shine and Lal, 1977; Bessman and Feinstein, 1979; Green and King, 1989; England, 1989; Lafferty et al, 1996; Eldibany et al, 1999; Ntaios et al, 2007; Sirdah et al, 2008), but other types of thalassemia traits, such as Hb E, α-thalassemia 1 and Hb CS (except α-thalassemia 2) traits, can also have microcytic RBC (Fucharoen et al, 1998b). A recent study, conducted in anemic vegetarian patients from the northern part of Thailand, demonstrated that a number of previously published formulae can be used to differentiate IDA from thalassemia traits, including both α- and β-thalassemia, but the highest AUC is 0.73 (Wongprachum et al, 2012).

The present study, performed in school-age students with no medical illnesses prior to the time of study, was able to determine an IDA incidence rate of 12%, similar to a previous report (Black et al, 2008). This study demonstrated that 57% of IDA subjects had thalassemia traits or
Table 1

Red blood cell parameters of normal, iron deficiency anemia (IDA), iron deficient erythropoeisis (IDE) and thalassemia trait subjects.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Hb (g/dl)</th>
<th>Hct (%)</th>
<th>RBC (x10⁶/µl)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dl)</th>
<th>RDW (%)</th>
<th>Ferritin (mg/ml)</th>
<th>Serum iron (µg/dl)</th>
<th>TIBC (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n = 108)</td>
<td>13.0(0.9)</td>
<td>39(2.7)</td>
<td>4.7(0.4)</td>
<td>84.5(4.9)</td>
<td>27.9(1.9)</td>
<td>33.0(0.6)</td>
<td>13.8(1.1)</td>
<td>45.0(13-188)</td>
<td>75.8(23.6)</td>
<td>329.1(52.2)</td>
</tr>
<tr>
<td>IDE (n = 99)</td>
<td>13.1(0.7)</td>
<td>39.4(2.0)</td>
<td>4.8(0.4)</td>
<td>82.6(4.8)</td>
<td>27.3(1.9)</td>
<td>33.0(0.6)</td>
<td>15.5(1.3)</td>
<td>35.3(3-208)</td>
<td>40.4(13.6)</td>
<td>356.9(51.2)</td>
</tr>
<tr>
<td>IDE with thalassemia trait (n = 35)</td>
<td>12.8(0.6)</td>
<td>39.3(2.0)</td>
<td>5.1(0.4)</td>
<td>76.8(1.2)</td>
<td>25.0(1.9)</td>
<td>32.6(0.6)</td>
<td>14.6(1.2)</td>
<td>25.2(7-268)</td>
<td>44.3(13.6)</td>
<td>373.2(66.1)</td>
</tr>
<tr>
<td>IDA (n = 17)</td>
<td>11.2(0.8)</td>
<td>35.0(1.9)</td>
<td>4.75(0.4)</td>
<td>74.3(7.9)</td>
<td>24.4(2.8)</td>
<td>31.4(3.5)</td>
<td>16.1(2.2)</td>
<td>34.6(3-100)</td>
<td>26.8(12.3)</td>
<td>352.6(74.6)</td>
</tr>
<tr>
<td>IDA with thalassemia trait (n = 23)</td>
<td>11.2(0.7)</td>
<td>35.1(1.8)</td>
<td>5.09(0.6)</td>
<td>69.7(7.9)</td>
<td>22.2(2.8)</td>
<td>31.8(0.8)</td>
<td>16.0(2.8)</td>
<td>38.1(3-162)</td>
<td>37.7(9.9)</td>
<td>340.2(46.2)</td>
</tr>
<tr>
<td>E trait (n = 29)</td>
<td>12.8(0.8)</td>
<td>37.8(7.0)</td>
<td>5.1(0.3)</td>
<td>77.0(4.0)</td>
<td>25.3(1.4)</td>
<td>31.8(5.5)</td>
<td>14.3(0.9)</td>
<td>51.4(20-233)</td>
<td>76.1(19.8)</td>
<td>334.7(37.1)</td>
</tr>
<tr>
<td>β-thalassemia trait (n = 10)</td>
<td>12.1(1.1)</td>
<td>37.4(2.5)</td>
<td>5.13(0.5)</td>
<td>73.7(9.7)</td>
<td>23.9(3.9)</td>
<td>29.3(9.1)</td>
<td>16.1(3.4)</td>
<td>47.5(22-90)</td>
<td>80.8(20.6)</td>
<td>317.4(43.8)</td>
</tr>
<tr>
<td>α-thalassemia 1, Hb CS</td>
<td>12.2(0.8)</td>
<td>37.9(2.0)</td>
<td>5.4(0.5)</td>
<td>70.5(6.2)</td>
<td>22.6(2.1)</td>
<td>32.1(0.8)</td>
<td>15.3(1.3)</td>
<td>42.9(27-160)</td>
<td>78.2(21.6)</td>
<td>328.0(54.8)</td>
</tr>
</tbody>
</table>
| Hb E+α-thalassemia 1 and Hb E +CS traits (n = 17) | Number is mean(±SD). Hb, hemoglobin; Hct, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; Plt, platelet; RBC, red blood cell; RDW, red cell distribution width; TIBC, total iron binding capacity.
new Formula to Differentiate Thalassemia and IDA

Table 2
Differences in red cell parameters and platelet counts between iron deficiency anemia (IDA) and thalassemia trait.

<table>
<thead>
<tr>
<th>RBC index</th>
<th>IDA(n = 40)</th>
<th>Thalassemia trait(n = 91)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>11.2 ± 0.7</td>
<td>12.6 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>35.0 ± 1.8</td>
<td>38.8 ± 2.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RBC (x10⁶/µl)</td>
<td>4.9 ± 0.5</td>
<td>5.2 ± 0.4</td>
<td>0.01</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>71.6 ± 8.1</td>
<td>75.3 ± 6.3</td>
<td>0.013</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>23.0 ± 2.9</td>
<td>24.5 ± 2.4</td>
<td>0.007</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>31.6 ± 2.3</td>
<td>32.2 ± 3.1</td>
<td>0.25</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>16.1 ± 2.5</td>
<td>14.8 ± 1.6</td>
<td>0.006</td>
</tr>
<tr>
<td>Platelet (x10³/µl)</td>
<td>395 ± 88.3</td>
<td>337 ± 75.7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Number is mean ± SD. Hb, hemoglobin; Hct, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell; RDW, red cell distribution width.

Table 3
Evaluation of formulae differentiating iron deficiency anemia and thalassemia trait.

<table>
<thead>
<tr>
<th>RBC index and formula</th>
<th>Equation</th>
<th>Cut-off of published value</th>
<th>AUC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Our formula</td>
<td>1.5xHb-0.05 MCV</td>
<td>&gt;14</td>
<td>0.92 (0.88-0.97)</td>
</tr>
<tr>
<td>RDW/RBC</td>
<td>RDW/RBC</td>
<td>&lt;3.3</td>
<td>0.81 (0.73-0.88)</td>
</tr>
<tr>
<td>Green and King formula</td>
<td>MCV²xRDW/Hbx100</td>
<td>&lt;72</td>
<td>0.68 (0.58-0.78)</td>
</tr>
<tr>
<td>England formula</td>
<td>MCV-RBC-5Hb-3.4</td>
<td>&lt;0</td>
<td>0.66 (0.55-0.76)</td>
</tr>
<tr>
<td>RDWI</td>
<td>RDWxMCH/RBC</td>
<td>&lt;220</td>
<td>0.64 (0.54-0.74)</td>
</tr>
<tr>
<td>Sridah M formula</td>
<td>MCV-RBC-3Hb</td>
<td>&lt;27</td>
<td>0.53 (0.42-0.64)</td>
</tr>
<tr>
<td>Mentzer Index</td>
<td>MCV/RBC</td>
<td>&lt;13</td>
<td>0.50 (0.44-0.76)</td>
</tr>
<tr>
<td>Srivastava formula</td>
<td>MCHC/RBC</td>
<td>&lt;3.8</td>
<td>0.49 (0.38-0.61)</td>
</tr>
<tr>
<td>Ehsani formula</td>
<td>MCV-10xRBC</td>
<td>&lt;15</td>
<td>0.46 (0.35-0.58)</td>
</tr>
<tr>
<td>Shine-Lal formula</td>
<td>MCV²xMCH/100</td>
<td>&lt;1,530</td>
<td>0.37 (0.26-0.47)</td>
</tr>
<tr>
<td>Bessman index</td>
<td>RDW</td>
<td>&gt;17</td>
<td>0.34 (0.23-0.46)</td>
</tr>
</tbody>
</table>

The cut-off values shown in the table are in favor of thalassemia trait. AUC, area under ROC curve; CI, confidence interval; Hb, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell; RDW, red cell distribution width.

The most common thalassemia trait was Hb E, followed by α-thalassemia 1 and β-thalassemia traits. DNA study for α-thalassemia 2 trait was not performed because it does cause microcytic RBC. High prevalence rates of both IDA and thalassemia traits have been previously reported (Madan et al, 1999; Nuchprayoon et al, 2003). Subjects with both conditions should firstly be treated with iron supple-
Source of the curve

- England and Fraser, 1973
- Sirdah et al, 2008
- Mentzer, 1973
- Ehsani et al, 2005
- Shine and Lal, 1977
- RDW/RBC
- Srivastava and Bevington, 1973
- RDW
- Green and King, 1989
- Our formula

**Fig 1–Area under receiver operative characteristic curves of the new formula and other formulae.**

ment, because IDA can cause cognitive impairment, especially in a young age group (Grantham-McGregor and Ani, 2001). If microcytic RBC still persists after iron treatment, thalassemia investigation should be performed (Nuchprayoon et al, 2003).

In order to come up with a formula to differentiate between the two conditions, the 10 previously reported formulae, which included RDW/RBC and RDW indices (England and Fraser, 1973; Mentzer, 1973; Srivastava and Bevington, 1973; Shine and Lal, 1977; Bessman and Feinstein, 1979; Ricerca et al, 1987; England, 1989; Green and King, 1989; Lafferty et al, 1996; Eldibany et al, 1999; Ehsani et al, 2005; Ntaios et al, 2007; Sirdah et al, 2008) were applied to differentiate IDA from thalassemia traits in our subjects who presented with microcytic RBC, but no formula had an AUC with acceptable sensitivity and specificity. This might be due to the fact that previous formulæ studied adults with mostly β-thalassemia trait. Our formula of (1.5 Hb - 0.05 MCV >14) had the highest AUC with sensitivity and specificity of 84.6% and 87.5%, respectively. Therefore, a score >14 is suggestive of thalassemia trait, while a score ≤14 is suggestive of IDA. However, in IDA subjects, thalassemia traits cannot be ruled out, and the follow-ups of RBC indices following a complete course of iron treatment is recommended. The limitation of this formula is that it may be more appropriate in areas which share similarities in the types of thalassemia traits. Also, it is important to note that in cases of IDA indicated by this formula, thalassemia should be investigated in subjects who do not completely recover from iron treatment.

In summary, due to the high prevalence of both IDA and thalassemia traits in certain regions of the world, a history of inadequate iron intake and thalassemia in a patient’s family, along with initial CBC, may not be adequate for the diagnosis of these conditions. The formula of (1.5 Hb - 0.05 MCV >14) provides an additional method to differentiate IDA from thalassemia traits. The validity of this formula needs to be evaluated in a larger sample population.

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